

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



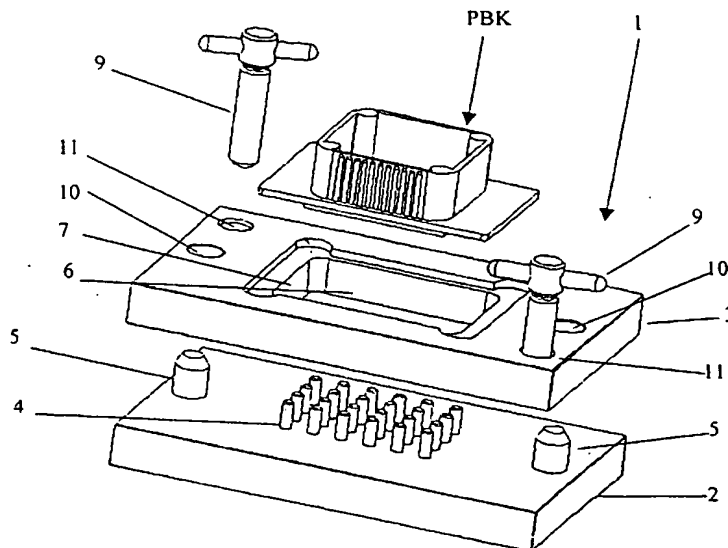
(43) International Publication Date  
31 December 2003 (31.12.2003)

PCT

(10) International Publication Number  
WO 2004/000992 A1

- (51) International Patent Classification<sup>7</sup>: C12M 1/00, G01N 1/36
- (21) International Application Number: PCT/HU2003/000044
- (22) International Filing Date: 17 June 2003 (17.06.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: U0200185 25 June 2002 (25.06.2002) HU
- (71) Applicants and  
(72) Inventors: SZEKERES, György [HU/HU]; Akác u. 8., H-7632 Pécs (HU). HALAS, Zsuzsanna [HU/HU]; Mária u. 16., H-7621 Pécs (HU). ZORN, Lászlóné [HU/HU]; Uitz B. u. 17., H-7632 Pécs (HU).
- (74) Common Representative: SZEKERES, György; Akác u. 8., H-7632 Pécs (HU).
- (81) Designated States (*national*): AU, CA, JP, US.
- (84) Designated States (*regional*): European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR).
- Declaration under Rule 4.17:**  
— of inventorship (Rule 4.17(iv)) for US only
- Published:**  
— with international search report  
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: TISSUE MICROARRAY BUILDER MANUAL SET



(57) Abstract: The tissue micro-array (TMA) building manual set, which includes a well-known SZK tissue core punch extractor and a TMA block mould (1) characterized as follows: the TMA block mould (1) consists of a lower (2) and an upper (3) part, the lower part (2) is equipped with mandrels (4) at right angles to the base unit arranged in the middle into lines and columns, the upper part (3) has a margin-containing (7) cleavage (6), which receives the mandrels (4) when the TMA block mould (1) is assembled, the margin (7) and size of the cleavage (6) fit to the shape and size of the paraffin block holder (PBK), and the lower (2) and upper (3) parts are connected to each other with directing nipples (5) and threaded arms (9) fixed horizontally.

WO 2004/000992 A1

## TISSUE MICROARRAY BUILDER MANUAL SET

The object of our invention is a tissue micro-array (TMA) preparing manual apparatus set, which includes a tissue core punch extractor and a TMA block mould with lower and upper parts.

The tissue micro-array preparing manual apparatus set is suitable for making TMA series, which enables the fast and accurate "home-made" preparation of TMA series well-fitted to the traditional, routine histology laboratory conditions.

TMA technology can be applied for the determination of diagnostic markers, as well as, parameters on the aggressiveness and other features of malignant tumors.

TMA is very useful for retrospective studies, i.e. for the investigation of thousands of molecular biological markers in thousands of histological specimens stored in pathology laboratories.

Similar to the DNA-chip, which allows the analysis of the genome mutations and abnormal gene expressions, the tissue micro-arrays enable the parallel processing of biological specimens. By using only one TMA, researchers have the opportunity for simultaneous comparative examination of various molecular parameters (DNA, RNA molecular biological techniques and immunohistochemistry/immunocytochemistry of antigens) in histological specimens of several malignant tumor tissues.

Numerous monoclonal antibodies, which determine the genome and phenotype of intact and pathological (mainly malignant) cells, and are specific for novel antigen-determinants, are being developed and introduced for diagnostic and therapeutic applications (immunomorphology). Diagnostic measures based on genetic methods, which can identify certain segments of DNA and/or RNA, are increasingly used (molecular biology). Several laboratories are involved in evaluating their tissue reactivity and potential applicability in the most prevalent tumors.

As a consequence, this diagnostic technique indeed seems to be widely used.

In the diagnostic technique, histology specimens from different tumor tissues are embedded in properly arranged sets into paraffin for the preparation of TMA. The TMA block is cut giving the various tumor tissues as point-like items in the histological light microscopic sections. Since each TMA block may render hundreds of parallel, 3-4  $\mu\text{m}$  thick serial sections,

hundreds of molecular parameters can be examined on the same micro-array. Several tumor markers can be analyzed within weeks, while this would take months per tissue specimen using traditional method.

According to our current knowledge, reactions made on standard-size histological sections (from frozen and/or fixed-embedded tissues) and cytological samples (smears, sediments, imprints, cytocentrifugates, etc.) are useful for the identification of molecular markers of tumors and for the prediction of disease progression. At the same time, merely limited number of samples can be examined due to technical and financial reasons, the evaluation is time-consuming, and comparative, as well as, reproducible examinations among different laboratories are difficult to carry out. First of all, the lack of reproducibility is the disadvantage of the so-called "multi-tissue block" technique that contains parts from various tissues/blocks in a simple histological block. Moreover, the distribution of individual molecular markers within the malignant tissue is often quite heterogeneous and, thus, a minute tissue part does not properly represent the biological features of the entire tumor cell population.

Application of the technique reminiscent of or rather based on the "chip" technology, i.e. the tissue micro-arrays, enables the investigation of hundreds or thousands of tissue specimens.

The knowledge in the field of medical biotechnological basic research is being multiplied, and its employment confirms both diagnostic and therapeutic measures. Nevertheless, the related applied research, research and development (R&D), as well as, clinical testing processes require years or even a decade and proportional financial means. Our invention, the TMA building manual set shortens the necessary testing phase in an accurate and cost-efficient way.

The information in the No. 4 820 504 US patent represents a well-known and widely used procedure, which serves for the preparation of a sample containing multiple tissue specimens. Its advantages include the simple procedure that can be applied in almost every laboratory equipped with routine and research facilities, low costs, and no need for procurement of new devices. However, the disadvantage of the technique is that the consecutive, side by side embedded tissue samples are randomly situated, not organized into lines and columns, which makes the evaluation rather difficult, the automatic (image analyzing) processing almost impossible or complicated and slow; the size of the individual tissue samples are merely roughly similar to each other. New instrument does not belong to the description of the procedure.

Our procedure and the object of our invention aims at addressing these disadvantages.

The No. 5 002 377 US patent is an instrument, which organizes tissue samples into regular lines and columns, and so gives a multiple tissue block. Its patent holder is the same as that of the previous instrument who is a well-known expert on this field, despite this technique has not become widely used. Its obvious advantage is that application of this instrument enables the preparation and embedding of regular, almost identical size tissue samples. Nevertheless, its disadvantage lies in that the individual tissue columns may be, though only slightly, shifted during block preparation. The largest disadvantages of the instrument are: its incompatibility with the most frequently applied and best known laboratory instruments (devices and semi-fixed assets) and, on the other hand, the need for three additional tools and the significant manual skills for their application. The first instrument includes multiple parallel cutting plates, which can cut out tissue columns from tissue samples, but can be applied exclusively on larger specimens. The other two components make possible the embedding of cut tissue columns into a single paraffin block.

The No. 6 103 518 US patent is a table device giving useful tissue micro-arrays. The procedure is based on the well-oriented technique of the recipient places of the paraffin block and the sampling from the selected sites of donor blocks (tissue cores). Besides its aforementioned advantages, its high price, complicated mechanical settings, and the need for one-by-one preparation of recipient holes of the recipient block represent serious drawbacks in the daily practice.

The objective of our invention is to eliminate the disadvantages of the above mentioned methods and to design a tissue micro-array preparing manual apparatus set that is simple, cheap, and compatible with all widely used histological laboratory fixed and semi-fixed assets. The principle of applying the manual apparatus is that the preparation of the recipient block is separated from the sampling; therefore, the samples can be previously prepared and stored elsewhere, then put into the recipient block. The manual apparatus requires small space, is easy to clean, there is basically no need for maintenance, each part is resistant to external physical and chemical impacts, and the recipient block can be prepared in one step.

Our invention is based on the recognition that the tissue micro-array preparing manual apparatus set should be made from elements that fit together in their size and can be applied along with the instruments used under the traditional routine histological laboratory conditions. This tissue micro-array preparing manual apparatus set was developed to contain a tissue core punch extractor and a TMA block mould with lower and upper parts, and the

traditional, most frequently used paraffin block holders fit well to this latter one. The tissue core punch extractor is a well-known technical solution, i.e. its function is based on punching. The punching tip is made as a punching tube with an internal diameter of 1 or 2 mm. This punching tube receives the tissue cylinder (core) taken from a given place of the sample (donor block), which can be manually pushed out from the punching tip by help of a piston-like needle probe pushed back by a coil spring inside the instrument. The external diameter of the needle probe is comparable to the internal diameter of the punching tip, but due to its size it can freely slide within the punching tip.

The TMA block mould is made up of two main elements: the lower and upper parts. The lower part is equipped with 1 or 2 mm wide mandrels, identical with the diameter of the punching tube, organized into lines and columns. Since the lower and upper parts are assembled together during the casting of the paraffin block and the paraffin is poured into the hole formed by the upper part, a margin should have been prepared there, which is identical in its size and shape with the size and shape of the generally used paraffin block holder. At the lower part at least two vertical directing nipple are built into the base unit, which ensures the parallel and intact removal of the paraffin block when the mould manual apparatus is taken apart. The equal withdrawal of the two elements at the opening of the lower and upper part of the TMA block mould is ensured by at least two diagonally arranged threaded arms.

The elimination of the above discussed disadvantages is ensured by a tissue micro-array preparing manual apparatus set with the following characteristics: a by itself already known tissue core punch extractor and a TMA block mould, which latter has a lower and upper part; the lower part is organized in the middle into lines and columns, set up with mandrels at right angles to the base unit; the upper part is prepared with a margin-containing cleavage, which receives the mandrels when the TMA block mould is assembled together; the margin and size of the cleavage fit to the shape and size of the paraffin block holder; the lower and upper parts are connected to each other with directing nipples and threaded arms fixed horizontally; the lower part of the TMA block mould has at least two vertical directing nipples, which vertically detachably join into the bore-holes of the upper part; in the upper part of the TMA block mould there are at least two diagonal transit bore-holes, which receive the threaded arms and the endings of the threaded arms sit on the lower part when the TMA block mould is assembled together.

Our invention, the TMA building manual set is presented in detail by means of drawings.

Figure 1. Axonometric delineation of the TMA block mould.

Figure 2. Axonometric delineation of the TMA block mould and paraffin block holder of the TMA building manual set.

Figure 3. The finished paraffin block with the tissue core punch extractor.

The 1 TMA block mould shown on Figure 1. is made up of two major elements, i.e. 2 lower part and 3 upper part. The 2 lower part has 4 mandrels with 1 or 2 mm diameter arranged into lines and columns. The diameter of the 4 mandrel is identical with the diameter of the 8 punching tube of the SZK tissue core punch extractor. On the 2 lower part there are 5 directing nipples at right angles to the lower part, which ensure the parallel and intact removal of the paraffin block when the 1 TMA block mould is dismantled. The size of the 3 upper part is identical with that of the 2 lower part and in the middle it has a 7 margin-containing 6 cleavage, which receives the 4 mandrels. The 7 margin of the 6 cleavage is made to receive the commercially available PBK paraffin block holder shown on Figure 2. On the 2 lower part there are 5 directing nipples, which join the 10 transit bore-holes on the 3 upper part. Moreover, the additional 11 transit bore-holes on the 3 upper part receives the 9 threaded arms, which ensure the even and parallel opening of the two parts of the assembled instrument cast with paraffin block.

The equal withdrawal of the two elements at the opening of the 2 lower and 3 upper part of the 1 TMA block mould is ensured by at least two diagonally arranged 9 threaded arms. The SZK tissue core punch extractor ends in a 8 punching tube with an internal diameter of 1 or 2 mm.

The well-known SZK tissue core punch extractor receives the tissue cylinder taken from a given place of the sample (donor block), which can be manually pushed out from the 8 punching tube by help of a piston-like needle probe pushed back by a coil spring inside the instrument.

The external diameter of the needle probe is comparable to the internal diameter of the 8 punching tube, but due to its size it can freely slide within the 8 punching tip.

On preparing the paraffin block, the assembled 1 TMA block mould is filled with paraffin through the 6 cleavage. After the paraffin is set when cooled down and removed from the instrument, it gives a PB paraffin block (recipient block) containing 1 or 2 mm wide

cylindrical holes with parallel longitudinal axes organized into regular lines and columns. These holes receive the tissue cores having the same diameter taken out from the donor blocks by the SZK tissue core punch extractor, giving finally the TMA block.

As it can be seen from our description, our objective was accomplished by the development of our invention. The TMA building manual set is a simple, cheap manual instrument, which is easy to handle and clean, environment-friendly, and enables the fast and accurate preparation of TMA under the traditional, routine histological laboratory conditions.

**Invention patent claims**

1. The tissue micro-array (TMA) building manual set, which includes a well-known SZK tissue core punch extractor and a TMA block mould (1) characterized with: the TMA block mould (1) consists of a lower (2) and an upper (3) part, the lower part (2) is equipped with mandrels (4) at right angles to the base unit arranged in the middle into lines and columns, the upper part (3) has a margin-containing (7) cleavage (6), which receives the mandrels (4) when the TMA block mould (1) is assembled, the margin (7) and size of the cleavage (6) fit to the shape and size of the paraffin block holder (PBK), and the lower (2) and upper (3) parts are connected to each other with directing nipples (5) and threaded arms (9) fixed horizontally.
2. The manual apparatus set as in the claim point No. 1 is characterized with: the lower part (2) of the TMA block mould (1) has at least two vertical directing nipples (5), which join the bore-holes (10) of the upper part (3) and are in vertical direction freely movable.
3. The manual apparatus set as in the claim point No. 1 is characterized with: the upper part (3) of the TMA block mould (1) has at least two diagonal transmit bore-holes (11), which receive the threaded arms (9) sit on the lower part (2) when the TMA block mould (1) is assembled together.



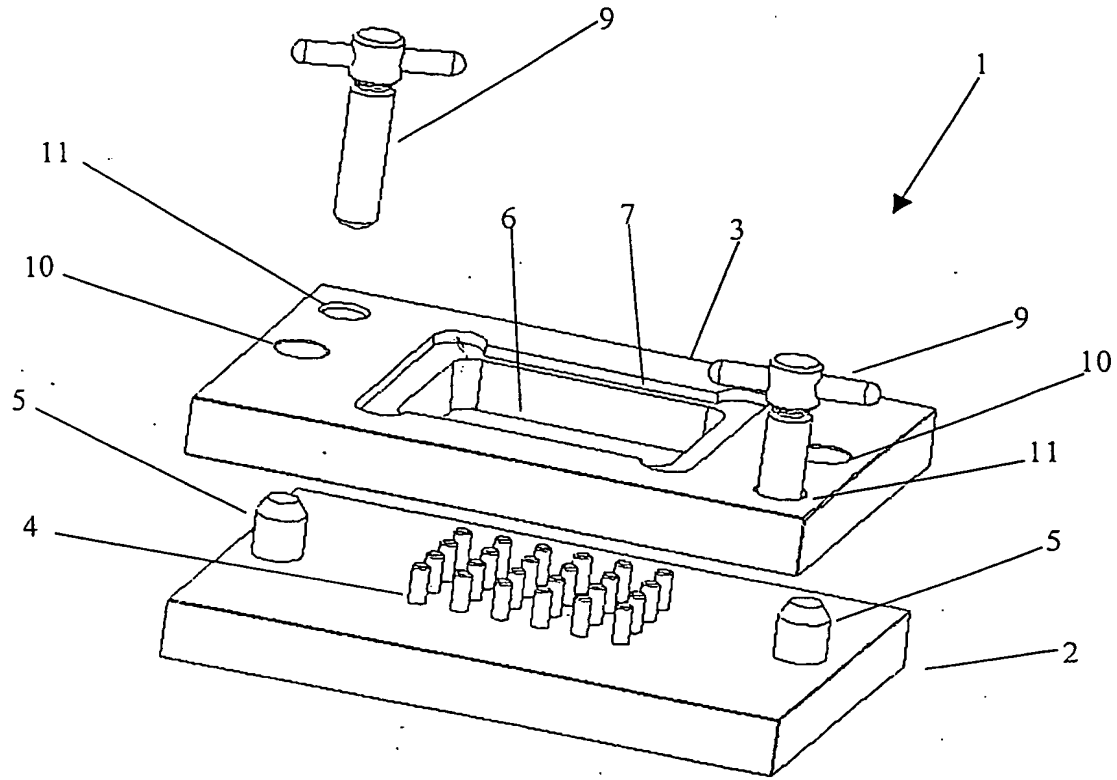


Figure 1

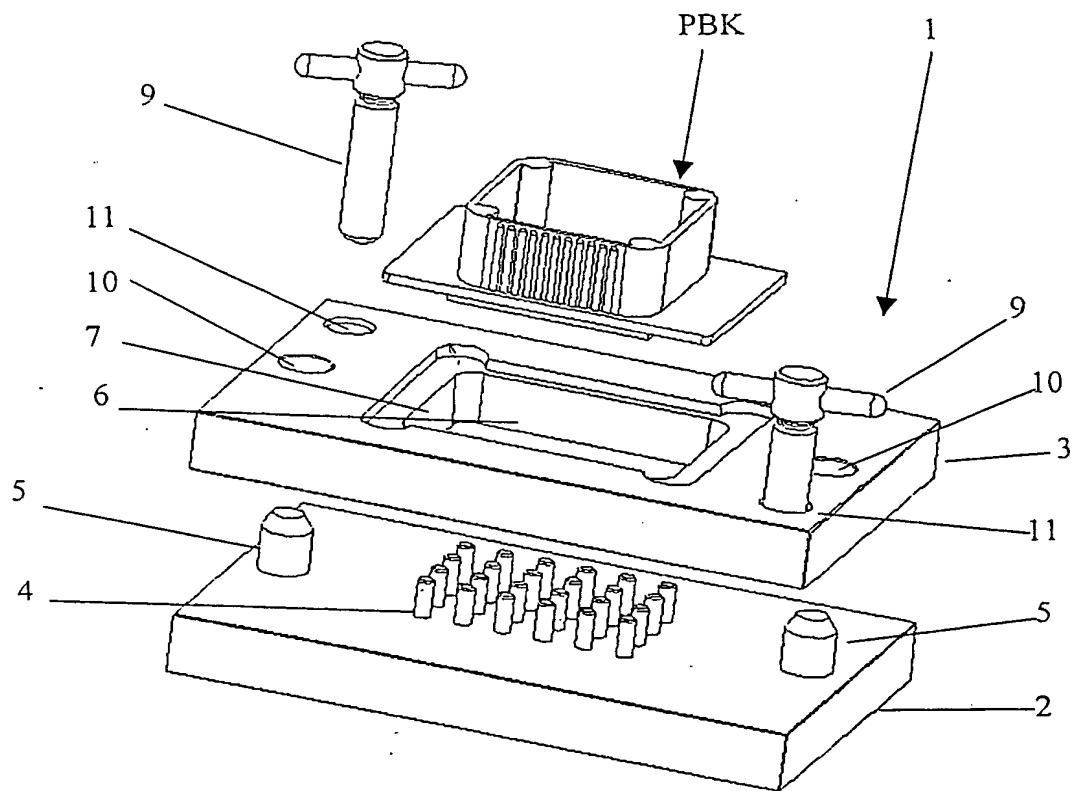


Figure 2

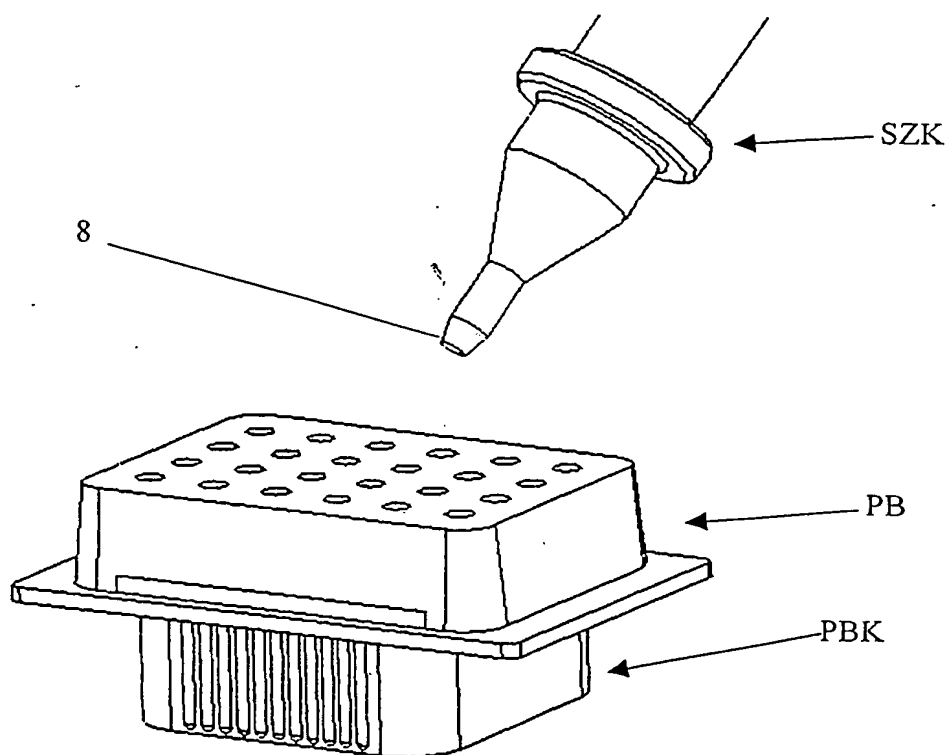


Figure 3

DT15 Re DT/PTO 27 DEC 2004

THIS PAGE BLANK (USPTO)